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RESEARCH PAPERS

Chinese medicinal plants: an alternative approach for management of Verticillium wilt of cotton

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Summary. The use of chemical fungicides is costly and potentially harmful to the environment. The trend towards the environmentally-friendly pesticides has led to the search for new antifungal agents from various sources, including Chinese medicinal herbs. This study aimed to confirm the antifungal potential of selected Chinese medicinal herbs, under *in vitro* and greenhouse conditions, against *Verticillium dahliae*, the causal agent of *Verticillium* wilt of cotton. Preliminary screening of 26 medicinal herbs for antifungal potential showed varied responses, reducing the radial colony growth of *V. dahliae*, and fungicidal potential. The strongest efficacy was observed for extracts from *Prunus mume* and *Rhus chinensis,* followed by *Coptis chinensis*, *Cortex phellodendri chinensis* (dried bark of *Phellodendron chinense*) and *Curcuma longa*. The efficacy of the four most effective herbs (*R. chinensis*, *P. mume, C. chinensis* and *C. phellodendri chinensis*) under greenhouse conditions correlated with *in vitro* tests. However, *Prunus mume* and *Rhus chinensis* gave the greatest reduction in severity of Verticillium wilt. The greatest improvements in plant height, fresh weight and number of leaves were obtained with *P. mume* and *R. chinensis*, followed by *C. chinensis* and *C. phellodendri chinensis*. Greatest improvements in plant growth resulted from *P. mume* and *R. chinensis*. Alternative control with Chinese medicinal herbs showing the greatest antifungal potential could provide economical, safe and non-hazardous tools for management of Verticillium wilt and increased cotton quality from sustainable production.

Key words: antifungal potential, *Verticillium dahliae.*

Introduction

Cotton (*Gossypium* spp.) is one of the most important cash crops grown worldwide, playing a key role in economic and social affairs. Generally, cotton is known as 'White gold' and is primarily grown in the tropical and sub-tropical regions worldwide. It is a leading natural fibre crop, as a source for textile industries throughout the world (Rahman *et al.*, 2008). This crop has major impacts on development of textile industries, generating employment and foreign exchange earnings for different countries. Conventionally, four out of 50 species of *Gossyipium* are used for cotton fibre production (Brubaker *et al.,* 1999). However, *G. hirsutum* and *G. barbadense* (tetraploids) are the principal species cultivated for fibre, representing, respectively, approx. 90 and 5% of worldwide production (Percy *et al.*, 2006). Cotton crops can be severely impacted by various factors, including Verticillium wilt.

Verticillium wilt, caused by the soil-borne pathogen *Verticillium dahliae* Kleb, is a challenging threat to sustainable cotton production throughout the world. Symptoms of this disease vary from host to host, and there are no common symptoms produced by *V. dahliae* in infected plants. The symptoms of Verticillium wilt are almost identical to those of Fusarium wilt. In many hosts and most areas, however, the

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pathogen induces wilt at lower temperatures than *Fusarium* spp. Moreover, the symptoms develop more slowly and often appear only on the lower or outer parts of host plants or on only a few of their branches (Agrios, 2005). *Verticillium dahliae* infects the roots of cotton plants and then gradually grows into the vascular systems (Pegg, 1989). In cotton, it mainly produces symptoms of marginal chlorosis and/or necrosis in leaves, wilting, discoloration of vascular bundles of stems, and decreases in photosynthesis and increases in respiration. Light to dark brown vascular discolouration is common in stems and branches of the infected cotton plants. The defoliating strains of the pathogen are the most virulent, causing typical wilt symptoms leading to the complete defoliation, gradual wilting and death of successive branches, or abrupt collapse and death of entire plants in severe cases. Significant reductions of plant biomass and heavy yield losses can ultimately result (Pegg, 1989; Hampton *et al.*, 1990; Paplomatas *et al.*, 1992; Agrios, 2005).

The use of resistant cotton germplasm is believed to be the most effective, economically feasible, and ecologically sustainable means of control of Verticillium wilt (El-Zik, 1985; Karademir *et al.*, 2010). However, presently in China there are only a few cotton lines which are resistant to *V. dahliae* (Khaskheli *et al.*, 2013), so there is a scarcity of highly resistant germplasm to manage and control this detrimental disease (Ma *et al.*, 2002). The use of fungicides for disease management (Tian *et al.*, 1998; Talboys, 1984) has been reported to be less effective, due to the persistent nature of fungus in the soil, and fungicides are also very costly and possibly detrimental to the environment (Berg *et al.*, 2001; Rekanovic *et al.*, 2007). In addition, continuous reliance on chemical control has led to economic losses and degradation of biodiversity. Alternative biocontrol agents are being developed for sustainable agriculture (Al-Rawahi and Hancock, 1998; Tenuta and Lazarovits, 2002; Aghighi *et al.*, 2004; Noble and Coventry, 2005; Huang *et al.*, 2006; Zheng *et al.*, 2011), to overcome the potential pollution and human health hazards resulting from the use of synthetic pesticides, and pathogen resistance to some pesticides (Spurrier, 1990; Tjamos *et al.*, 2000; Zheng *et al.*, 2011). However, the performance of biocontrol agents under unstable field conditions is not always consistent (Weller and Cook, 1983; Berg *et al.*, 2000). Consequently, it is challenging for producers to find effective disease control strategies (Junli *et al.*, 2006).

The trend towards environmentally friendly pesticides has led to searches for new antimicrobial agents from various sources, including medicinal plants (Aqsa *et al.*, 2010). Plants synthesize compounds with antibiotic and antimicrobial properties (Kumar and Parmar, 1996; Prakash and Rao, 1997; Amadioha, 2003; Opara and Wokocha, 2008). Such compounds can be exploited for alternative disease management of Verticillium wilt. Use of pesticides of plant origin is likely to have minimal toxic effects to humans and other organisms, and is also likely to be environmentally safe, and these materials probably have low risks of development of resistance by pathogens (Kumar and Parmar, 1996; Prakash and Rao, 1997; Amadioha, 2003). Biopesticides may also be less expensive, easily available (because of their natural occurrence) and should not affect seed viability, plant growth or food quality (Loper *et al.*, 1991; Opara and Wokocha, 2008; Aqsa *et al.*, 2010; Ernest *et al.*, 2012).

Biological screening of plant extracts is carried out throughout the world for determination of antifungal activities (Aqsa *et al.*, 2010). Several medicinal plants have antimicrobial activity against different pathogens (Bowers and Locke, 2000; Bowers and Locke, 2004; Muto *et al.*, 2005; Hassanein *et al.*, 2008; Aqsa *et al.*, 2010; Lakhdar *et al.*, 2010; Zaker and Mosallanjad, 2010). Most Chinese medicines consist of herbs, and some of these have been used for treating microbial infections (Huang, 1991; Bensky and Gamble, 1993; Wang *et al.*, 2006). Lopez-Escudero *et al.* (2007) reported reduction of viability of *V. dahliae* microsclerotia in soil by dried plant residues. *In vitro* inhibition *V. dahliae* with different extracts of Chinese traditional medicine has also been previously reported (Zhu *et al.*, 2007).

Plant based pesticides which are relatively economical, safe and non-hazardous, can therefore be used successfully against the plant pathogenic fungi including *V. dahliae*. The present study aimed to confirm the antifungal potential against *V. dahliae* of selected Chinese medicinal herbs, using *in vitro* and greenhouse evaluations. This research also aimed to evaluate these plants for potential use as sustainable and eco-friendly integrated disease management tolls for cotton crops, and for farmers and managers.

Materials and methods

Host plant material

In vitro and greenhouse experiments were conducted twice during two successive years, 2011 and

2012. Host cotton plant germplasm Arcot-402bne (very susceptible to Verticillium wilt) was used in these experiments.

Source of Chinese medicinal herbs

Twenty-six Chinese medicinal herbs were used (Table 1), and these were obtained from a Chinese herb store, TianDa Pharmacy, in Anyang, Henan, Peoples' Republic of China.

Pathogen inoculum

The inoculum of highly virulent strain D07073-3 (Defoliating) of *V. dahliae* obtained from the Plant Protection Department, Institute of Cotton Research

Table 1. List of the Chinese medicinal herbs used in this study.

Treatment	Botanical name	Common name	Tested plant part
T-1	*Azadirachta indica* A. Juss	Neem	Seeds
T-2	*Allium sativum* L.	Garlic	Bulb
T-3	*Capsicum annuum* L.	Chile/pepper	Fruit
T-4	*Perilla frutesces* L. (*Caulis perillae*)	Perilla stem	Stem and bark
T-5	*Curcuma longa* L.	Turmeric	Fruit
T-6	*Ginkgo biloba* L.	Maidenhair tree	Fruit
T-7	*Cinnamomum cassia* Nees & T. Nees	Cassia, Chinese cinnamon	Bark
T-8	*Astragalus propinquus* Schischkin	Chinese astragalus	Bark
T-9	*Polygonum multiflorum* Thunb	Knotweed, knotgrass, bistort	Bark/leaves
T-10	*Perilla frutescens* L.	Shiso	Leaves and bark
T-11	*Melia azedaeach* L.	Chinaberry tree, bead-tree	Bark
T-12	*Rhus chinensis* Mill.	China sumac,	Fruit
T-13	*Cnidium monnieri* (L.) *Cuss*	Common cnidium fruit	Fruit
T-14	*Solanum lyratum* Thunb	Night shade	Stem/bark
T-15	*Ephedra sinica* Stapf	Herba Ephedrae	Stem/bark
T-16	*Cortex phellodendri chinensis*[a]	Amur corktree bark	Bark
T-17	*Tripterygium wilfordii* Hook. f.	Three wing nut root	Root
T-18	*Perilla frutescens*	Perilla	Stem/fruit
T-19	*Curcuma aromatica* Salisb.	Wild turmeric	Fruit
T-20	*Prunus mume* Siebold & Zucc. Siebold and Zuccarini	Dark plum fruit	Fruit
T-21	*Clematis chinensis* Osbeck	Wei ling xian in Chinese	
T-22	*Magnolia liliiflora* Desr	Lily magnolia/ magnolia wood	Bark
T-23	*Rhizoma Polygoni* Cuspidati	Tiger stick, Japan fleece flower, giant knotweed	Bark
T-24	*Angelica dahurica* (Fisch.) Benth. et Hook	Dahurica angelica root	Root
T-25	*Coptis chinensis* Franch	Short sepal goldthread	Bark/ stem
T-26	*Aloe vera* (L.) Burm.f.	Aloe vera	Extract

[a] *Cortex phellodendri chinensis* is derived from the dried bark of *Phellodendron chinense* Schneid.

of the Chinese Academy of Agricultural Sciences (ICR.CAAS), Anyang, Henan Province, was used in the present study. This fungus was grown on potato dextrose agar (PDA) or Czapek-Dox broth (DIFCO). For PDA culture, a 5 mm diam. mycelial plug of freshly grown *V. dahliae* was transferred to each PDA plate, and plates were incubated at 25°C for 24–25 d prior to inoculation. For the liquid cultures, three mycelial plugs (5 mm diam.) were used separately to inoculate 150 mL of medium in each 250 mL capacity Erlenmeyer flask. The liquid cultures were grown on a rotary shaker at 25 ± 1°C for 7–8 d. The fungus suspension was then passed through double-layered cheesecloth to remove mycelial fragments. The microconidia concentration was determined using a haemocytometer, and appropriate dilutions were made to adjust the inoculum to 10^7 microconidia mL^{-1} concentration with sterile distilled water. The resulting suspensions were used to inoculate the plants using soil drenches (100 mL microconidium suspension per 2 kg soil).

Preparation of stock solutions

All the Chinese medicinal herbs were oven dried at 60°C for 10 min. After drying, each herb was ground and crushed through a grinding mill. The mill was cleaned with water and then alcohol between operations for material from the different herbs. Ten grams of each dried powder was added to a 100 mL capacity beaker containing 80 mL of distilled water, and left for 3 h at room temperature. Each suspension was then heated to boiling for 10 min, and then reduced to 20 mL of filtrate. This extract was filtered through two layers of muslin cloth and the resulting solution was autoclaved at 121°C for 20 min. The filtrates were then used as stock solutions (Zhu *et al.*, 2007).

In vitro antifungal tests

The preliminary antifungal screening against *V. dahliae* of 26 Chinese herbs was carried out under laboratory conditions using Petri plate methods (Grover and Moore, 1962; Nene and Thapliyal, 1973; Mishra and Tiwari, 1992). The experiments were conducted twice in completely randomized designs with eight replications and three concentrations of each herb extract (0.01, 0.015 and 0.02 g mL^{-1}). Petri dishes containing amended medium were each inoculated with a 5 mm diam. disk of freshly prepared culture of *V. dahliae* under aseptic conditions. Un-amended PDA plates were used as experimental controls. All the inoculated plates were incubated at 25 ± 1°C. Colony growth was recorded after 10 and 30 d incubation, when growth in the control plates completely covered the medium. The proportional (%) inhibition of mycelial growth over controls was calculated using the formula of Vincent (1947): $I = (C-T)/C \times 100$; where, I = percent inhibition, C = radial colony growth in control, and T = radial colony growth in treatment.

Test of four selected Chinese herbs under greenhouse conditions

A series of experiments were each performed twice under greenhouse conditions to determine the effectiveness of treatments by three different application techniques (seed treatment + spray, seed treatment + drench, or seed treatment + drench + spray) to reduce Verticillium wilt development. Based on preliminary screening, the four Chinese herbs *Rhus chinensis* Mill, *Prunus mume* Siebold & Zucc., *Coptis chinensis* Franch, and *Cortex phellodendri chinensis*, were selected and applied at one concentration (2 mL L^{-1} stock solution). Treated and untreated seeds were sown in 15 × 12 cm plastic pots, with ten seeds of each cotton germplasm sown into each pot. The pots each contained 1 kg of autoclaved sandy soil (sand and soil, 1:3). The experiments were set out in randomized complete block designs with two factors being evaluated. These included Chinese herbs (treatments) and the application techniques. Plants were inoculated with *V. dahliae* using soil drench methods to evaluate the efficacy of the all treatments. For soil drenching, 100 mL of microconidia suspension (10^7 conidia mL^{-1}) was irrigated into each pot before sowing the seeds. Inoculated untreated soil was considered as one experimental control, and un-inoculated untreated soil was used as a second control.

Seed treatments and application of Chinese herb extracts

Seeds of highly susceptible cotton germplasm were treated with extracts from four selected Chinese herbs. De-linted cotton seeds were dipped in the solutions of each extract treatment (8 mL of stock solution) suspended in 1.5% carboxy-methyl-cellulose (CMC) solution in sterile distilled water for 1 h, and then air dried under laminar air flow for seed the treatment (ST) prior to sowing. Seeds treated with CMC solution were used in control treatments. Plants were treated twice; firstly (8 mL for spray and drench) at 20 d after inoculation (DAI) and secondly at after a further 15 d. In the greenhouse, three different methods of treatments were applied: 1) Seed Treatment + Spray; 2) Seed Treatment + Drench and 3) Seed Treatment + Drench + Spray.

Assessment of treatment efficacy

The efficacy of treatments was assessed using different host growth parameters, percent wilt incidence (WI) and percent infection. Disease symptoms of Verticillium wilt were recorded in both germplasm lines as reported by Paplomatas *et al.* (1992) 15 d after each treatment. Individual plants that showed wilt symptoms were considered as diseased. Data were recorded for healthy and wilted plants, with wilt incidence (%) calculated using the formula: Wilt Incidence (WI) = (Number of wilted plants/Total number of observed plants) × 100. The efficacy of all treatments was calculated using the Abbott's formula (Abbot, 1925): Efficacy (%) = (X-Y)/X × 100; Where, X = disease severity of the control, and Y = disease severity of the treatment. Infection of plants by *V. dahliae* was verified by isolating the fungus from affected roots and shoots. Two plants were randomly collected and a general method for isolating fungi from plant tissues was utilized (Dhingra and Sinclair 1995). The infection rate and intensity of infection (%) were calculated using the formula: Infection (%) = (Number of pieces colonized by the fungus/ Total number of pieces) × 100. Plant growth parameters, including plant height (cm), plant weight (g), and the number of leaves, were also recorded. Six plants per replication were assessed for all parameters. Increases in biomass compared with experimental controls were calculated using the formula: Increase (%) compared with control = [(fresh weights of plants treated - fresh weights of control plants)/ fresh weights of control plants] × 100.

Statistical analyses

The *in vitro* and greenhouse experiments were repeated twice, and combined analyses of both trials are presented. Data were statistically analyzed according to standard procedures for analysis of variance, (ANOVA; linear model), and mean separation (least significant difference, LSD) of all parameters including wilt incidence (WI), efficacy, increase (%) over control (after calculating with corresponding formulae), and interactions between the trials, were calculated using the computer software Statistix 8.1 (Analytical Software 2005). All differences described in the text were statistically significant at the 5% level of probability.

Results

Antifungal potential of Chinese herbs against the *Verticillium dahliae*

The antifungal potential of 26 Chinese herbs (Table 1) tested in Petri plate assays demonstrated varied responses of reduced *V. dahliae* mycelial colony growth, and showed fungicidal effects. Analysis of variance indicated statistically significant differences among all the treatments and three doses of each, for reduced mycelial colony growth of *V. dahliae*. The treatment by dose interactions were also significant. However, no significant difference was observed between two trials (Table 2). All the tested treatments of Chinese herbs gave inhibitory effects. The greatest inhibition of *V. dahliae* mycelial growth was induced by extract from *Rhus chinensis* (61%) followed by *Prunus mume* (59%), *Coptis chinensis* (44%), *Cortex phellodendri chinensis* (31%) and *Curcuma longa* (29%). However, no significant difference was found between *Rhus chinensis* and *Coptis chinensis* at greater dose (Figure 1). The overall performance of the different Chinese medicinal herbs is illustrated in Figure 2.

Potential of extracts under greenhouse conditions

Antifungal activity

To analyse the antifungal potential of the herbs tested, four treatments of *Rhus chinensis*, *Prunus mume, Coptis chinensis* and *Cortex phellodendri chinensis* were tested for the reduction of Verticillium wilt based on the laboratory screening and test. Percent WI incidence of the tested cotton germplasm varied significantly among the different herbs assessed. There were also differences from the different methods of application, but no significant difference was observed for the two trials carried out (Table 2). The efficacy of the Chinese herb extracts tested to reduce the severity of Verticillium wilt under greenhouse conditions showed significant differences among

Table 2. Analyses of variance for effect of Chinese medicinal herbs on for *in vitro* (inhibition of mycelial growth) and greenhouse (WI, Efficacy, Infection, Germination, Mortality and Live Percentage) experiments.

Source	*In vitro* test		DF	WI (%)	Efficacy (%)	Infection (%)	DF	Mortality (%)	Live (%)
	DF	MS		MS				MS	
Treatment	26	7111.53**	5	29188.5**	33858.6**	9497.72**	4	14689.7**	14689.7**
Method	-	-	2	1694.42**	1959.0**	105.91**	2	227.1*	227.1*
Dose	2	17263.1**	-	-	-	-		-	-
Replication	7		4	196.778	347.3	12.10	3	128.0	128.0
Trial	1	18.7778	1	2.16000	1.6	0.20	1	2.4	2.4
Treatment × Dose	52	1311.64**	-	-	-	-		-	-
Treatment × Method		-	8	158.757*	183.6*	9.92	8	46.2	46.2
Treatment × Trial	26	0.02778	4	0.00375	0.1	0.01	4	1.0	1.0
Error	1188	16.2208	126	64.0282	61.0	5.50	97	42.4	42.4
Total	1295	-	149	-			119		
CV% (P<0.05)	-	15.96		19.10	14.22	15.52		26.08	8.68

* Significant
** Highly significant
DF, Degree of freedom
MS, Mean square
WI, Wilt Incidence

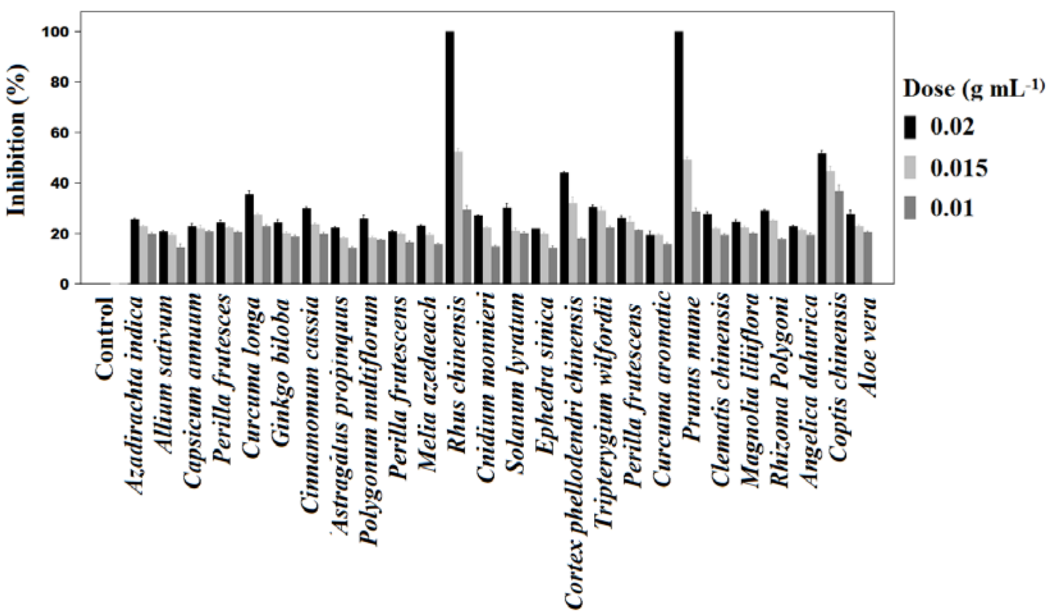

Figure 1. Mean inhibition percentages of mycelial colony growth of *Verticillium dahliae* by extracts of different Chinese medicinal herbs, under *in vitro* conditions.

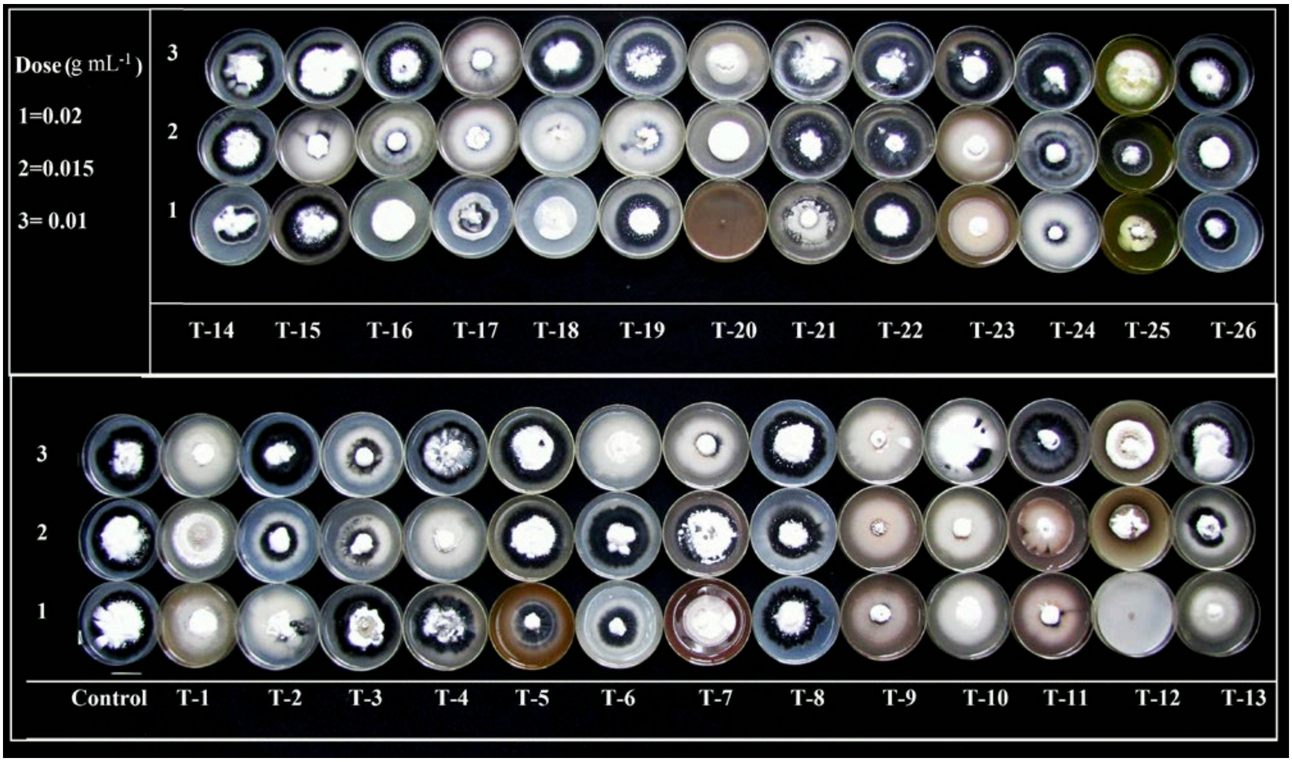

Figure 2. Performance of extracts from different Chinese medicinal herbs (see Table 1) on inhibition mycelial colony growth of *Verticillium dahliae* under *in vitro* conditions.

four treatments (*Rhus chinensis*, *Prunus mume, Coptis chinensis*, and *Cortex phellodendri chinensis*). *Prunus mume* showed the greatest efficacy (84%), to reduce Verticillium wilt severity by 15%, followed by *Rhus chinensis* (77%) by 21%. However, *Coptis chinensis* followed by *Cortex phellodendri chinensis* performed moderately compared to *Prunus mume* and *Rhus chinensis* for reducing Verticillium wilt severity (32 and 48%, respectively; Figure 3b).

Figure 3a also presents the WI percentage for different application methods used to treat the susceptible cotton germplasm under greenhouse conditions. The lowest WI percentage was recorded with Seed Treatment + Drench + Spray, compared to Seed Treatment + Spray; and Seed Treatment + Drench, for all four treatments. Similarly, lower *V. dahlia* infection rate was observed from the *Prunus mume* extract, (4%) followed by *Rhus chinensis* (5%), showing the greatest efficacy.

Figure 3c presents the data for re-isolation of *V. dahliae* from plant tissues following treatments with extracts from four Chinese medicinal plants. Extracts from *Prunus mume* and *Rhus chinensis* produced the lowest infection percentage compared to the controls. However, *Coptis chinensis* followed by *Cortex phellodendri chinensis* also inhibited the development of *V. dahliae* (Figure 3c).

Chinese herb extracts gave significantly different effects ($P<0.05$) on mortality and living (%) of cotton plants under greenhouse conditions. There was no significant difference between two trials in this study (Table 2). Greatest plant mortality (25%) was recorded for the *Cortex phellodendri chinensis* treatment, followed by *Coptis chinensis* (15%) (Figure 4a). However, the maximum percentage of surviving plants were those treated with *Rhus chinensis* (92%) and *Prunus mume* (90%) (Figure 4b).

Plant growth promotion

Plant growth parameters (height, fresh weight and number of leaves) of cotton plants treated with different Chinese herbs under greenhouse conditions were different for the different treatments. However,

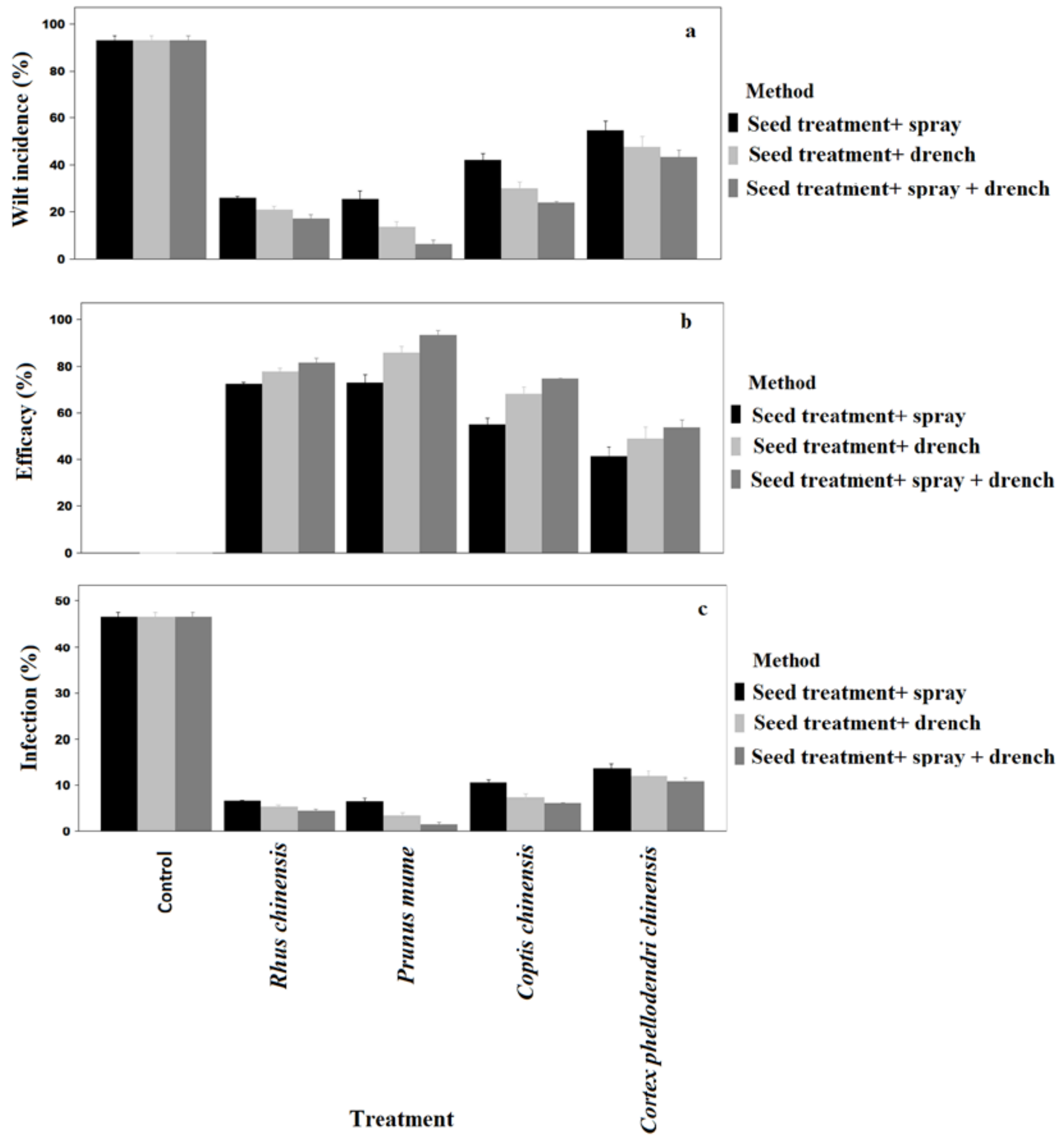

Figure 3. Mean wilt incidence (a), efficacy (b) and infection percentage (c) of susceptible cotton germplasm treated with extracts from four Chinese medicinal herbs applied with three different treatment methods, under greenhouse conditions.

the different methods of treatment gave different effects. Plant height and fresh weight varied, but there was no effect on numbers of leaves (Table 3).

Significant increases in plant height resulted from treatments with *Rhus chinensis*, *Prunus mume* (both 32% increase) followed by *Cortex phellodendri chinensis* (28%) and *Coptis chinensis* (27%). The greatest increase in plant fresh weigh over control was observed for *Prunus mume* (71%) followed by *Rhus chinensis* (70%), *Cortex phellodendri chinensis* (69%) and *Coptis chinensis* (69%). However, no significant difference was recorded for *Cortex phellodendri chinensis* and *Coptis chinensis* (Table 4). Significantly greater increases (%) over controls for number of leaves per plant resulted from treatment with extracts from *Prunus mume* (76% increase), *Rhus chinensis* (77%), *Cortex phellodendri chinensis* (75%) and *Coptis chinensis* (74%). However, no significant difference was

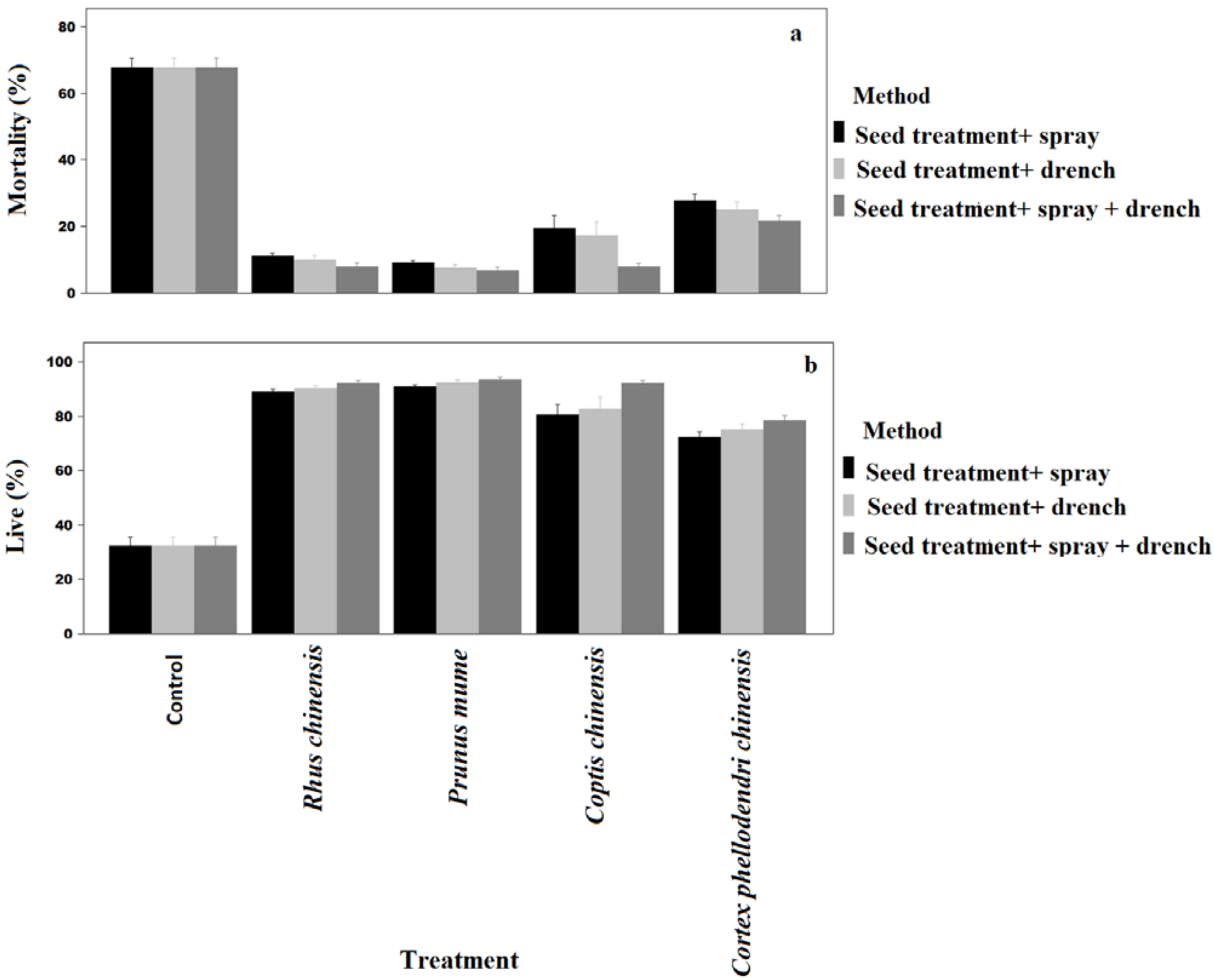

Figure 4. Mean proportions of dead and living cotton plants under greenhouse conditions, after treatment with extracts from different Chinese medicinal herbs, applied using three different methods.

recorded for *Cortex phellodendri chinensis* and *Coptis chinensis* (Table 4).

Different methods of treatment also influenced the efficacy of the plant extracts. Significantly lower WI percentages were recorded from the Seed Treatment + Drench + Spray applications, followed by Seed Treatment + Spray for all treatments. The efficacy of *Prunus mume* (93%) and *Rhus chinensis* (81%) was greater than *Coptis chinensis* (74%) and *Cortex phellodendri chinensis* (54%). However, *Prunus mume* (86%) also showed significantly greater efficacy from Seed Treatment + Spray compared to the other treatments.

Discussion

Several studies have shown that organic pesticides (of plant origin) can control plant diseases, and these products have minimal toxicity to humans and other organisms, and are also environmentally safe (Kumar and Parmar, 1996; Prakash and Rao, 1997; Amadioha, 2003). On the contrary, the use of chemical fungicides is costly and they can be can be toxic and environmentally harmful (Berg *et al.*, 2001; Rekanovic *et al.*, 2007). In addition, continuous reliance on chemical control has led to losses and degradation of biodiversity. Thus, the

Table 3. Analyses of variance for plant growth parameters influenced by extract from four Chinese herbs under greenhouse conditions

Source	DF	Plant growth (MS)			Increase (%) over control (MS)		
		Height	Weight	No. of leaves	Height	Weight	No. of leaves
Treatment	4	527.348**	13.2113**	185.493**	6468.94**	35065.34**	41004.6**
Method	2	13.824**	0.0426**	0.056	50.26**	7.54**	0.77144
Replicas	5	0.800	0.0085	0.920	3.23	0.3	9.51974
Trial	1	3.200	0.4898	0.072	65.97	11.6	3.28320
Treatment × Method	8	0.864*	0.0024	0.003	3.19	0.5	0.04968
Treatment × Trial	4	2.575	0.0485	0.067	4.18	14.7	0.27840
Error	155	0.413	0.0012	0.222	2.08	0.3	2.10178
Total	179						
CV		2.43	2.09	8.26	6.08	0.96	2.40

* Significant
** Highly significant
DF, Degree of freedom
MS, Mean square
CV, Coefficient of variance

trend towards the environmentally friendly pesticides for pests that are resistant to commonly used pesticides has led to the search for new antimicrobial agents from various sources, including medicinal plants (Aqsa *et al.*, 2010). Plants synthesize compounds with antibiotic and antimicrobial properties (Kumar and Parmar, 1996; Prakash and Rao, 1997; Amadioha, 2003; Opara and Wokocha, 2008). These can be exploited as alternative approaches to control of Verticillium wilt in cotton. Plant based pesticides which are relatively cheap and safe can be used successfully against *V. dahliae*.

Antifungal potential of Chinese herbs against the *Verticillium dahliae*

This study screened 26 Chinese medicinal herbs under *in vitro* conditions to assess antifungal potential against *V. dahliae*. The preliminary screening of extracts from these plants showed varied responses for reduced colony growth of *V. dahliae*, and showed antifungal potential. All the tested plants reduced *V. dahliae* colony growth. However, the efficacy was variable and could be increased by manipulating the concentration of plant extracts used. Greatest antifungal efficacy was measured for extracts from *Prunus mume* and *Rhus chinensis.* Similarly, the inhibitory effects of *Coptis chinensis*, *Cortex phellodendri chinensis* and *Curcuma longa* were greater than for the other Chinese medicinal herbs assessed. *In vitro* inhibition of *V. dahliae* with different Chinese traditional medicinal extracts has also been reported by Zhu *et al.*, (2007). Their results showed that the inhibition of greater than 45% occurred with extracts from China Sumac (*Rhus chinensis*), Common Cnidium fruit (*Cnidium monnieri* L. Cuss), Nightshade (*Solanum lyratum* Thunb), Herba Ephedrae (*Ephedra sinica* Stapf), Croton Fruit, Amur Corktree Bark (*Cortex phellodendri chinensis*) and Short sepal Goldthread (*Coptis chinensis*). Their further studies showed that China Sumac and Common Cnidium fruit gave greatest inhibition of *V. dahlia*. The *in vitro* results in the present study are in agreement with those of Zhu *et al.* (2007) regarding the strongest efficacy of China Sumac (*Rhus chinensis*); however, the performance of Common Cnidium Fruit and Nightshade were poor in our study. Similarly, Wang *et al.*, (2006) reported the best inhibitory extract from gallnuts of Chinese sumac against spore germination of *Alternaria bras-*

Table 4. Influence (percent increases over experimental controls) of four Chinese medicinal herbs (applied with three different treatment methods) on plant growth under greenhouse conditions. Control was inoculated but untreated.

Treatment	Seed treatment+ spray	Seed treatment + drench	Seed treatment + spray + drench	Mean
Plant height (cm)				
Rhus chinensis Mill	30.5 b[a]	31.8 a	32.5 a	31.5 a
Prunus mume Siebold & Zucc.	30.3 b	31.8 a	32.5 a	31.5 a
Coptis chinensis Franch	26.9 ef	28.5 cd	29.3 bc	28.2 b
Cortex phellodendri chinensis	26.0 f	27.6 de	28.4 cd	27.4 c
Control	0.00 g	0.00 g	0.0 g	0.00 d
SE	0.6	0.6	0.6	0.3
LSD P<0.05	1.16	1.16	1.16	0.67
Fresh plant weight (g)				
Rhus chinensis Mill	69.5 e	70.0 d	70.5 bc	70.0 b
Prunus mume Siebold & Zucc.	70.4 cd	70.8 b	71.3 a	70.8 a
Coptis chinensis Franch	68.6 f	69.2 e	69.4 e	69.0 c
Cortex phellodendri chinensis	68.7 f	69.2 e	69.5 e	69.1 c
Control	0.0 g	0.0 g	0.0 g	0.0 d
SE	0.2	0.2	0.2	0.1
LSD P<0.05	0.43	0.43	0.43	0.25
Leaves (No.)				
Rhus chinensis Mill	75.6 bcd	75.9 abc	75.9 abc	75.8 b
Prunus mume Siebold & Zucc.	76.7 ab	76.9 a	76.9 a	76.8 a
Coptis chinensis Franch	74.6 de	74.8 cde	74.9 cde	74.7 c
Cortex phellodendri chinensis	74.2 e	74.4 de	74.5 de	74.4 c
Control	0.0 f	0.0 f	0.0 f	0.0 d
SE	0.6	0.6	0.6	0.3
LSD P<0.05	1.16	1.16	1.16	0.67

SE, Standard Error

[a] Figures followed by the same letter within a column were not significantly different (least significant difference test (P=0.05).

sicicola, and also found this to be strongly inhibitory to *Colletotrichum gloeosporio-ides*, *Corynespora cassiicola*, *Fusarium oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *tracheiphilum*, *Phytophtho-ra capsici,* and *P. infestans*. This evidence indicates that *Rhus chinensis* has strong antifungal potential against different fungi including *V. dahliae*.

The present study also demonstrated strong efficacy of *Prunus mume, Coptis chinensis* and *Cortex phellodendri chinensis,* especially the antifungal potential of *Prunus mume,* and that this was almost the same as that of from *Rhus chinensis*. However, the present study also applied extracts of these four plants for the reduction of Verticillium wilt severity under greenhouse conditions. Correlation of results with laboratory efficacy indicates that these Chinese medicinal herbs could effectively reduce the development of *V. dahliae* in crop environments. Furthermore, there are no lines of evidence regarding the use of Chinese medicinal plant extracts under greenhouse conditions. The present study is a preliminary demonstration of the efficacy of these potent plant extracts, and further study is required to confirm their antifungal potential under field conditions.

Plant growth promotion

Biopesticides are in-expensive, easily available in nature (because of their natural occurrence), and, depending on concentration, may have no effects on seed viability, plant growth and food quality (Loper *et al.,* 1991; Opara and Wokocha, 2008; Aqsa *et al.,* 2010; Ernest *et al.,* 2012). Thus, biological screening of plant-based bio-products has been carried out worldwide to determine their antifungal activity. In the present study, four potential Chinese medicinal herbs were screened, which produced strong antifungal activity against *V. dahlia* as reduction of radial mycelial growth, along with reduced wilt incidence in greenhouse tests. The treatments also increased plant growth parameters compared to inoculated controls. It has been confirmed that the four Chinese herbs tested under greenhouse conditions were not toxic to plant growth at the tested concentrations. Treatment of cotton plants with extracts of *Prunus mume* or *Rhus chinensis* increased plant height, fresh weight and numbers of leaves under greenhouse conditions. Extracts from *Coptis chinensis* and *Cortex phellodendri chinensis* also increased cotton growth parameters. No significant differences in plant height for plants treated with *Prunus mume* or *Rhus chinensis* were detected. Similarly, no increases in plant fresh weights or numbers of leaves were measured for *Coptis chinensis* and *Cortex phellodendri chinensis* treatments. This indicates that the increase in plant growth may have resulted from manipulating the concentrations of two medicinal herbs, and efficacy against wilt can also be enhanced. The best Chinese medicinal herbs to improve plant growth were *Prunus mume* and *Rhus chinensis*.

Further study is required to determine the mechanism and function of antifungal potential of Chinese medicinal herbs. Knowledge of the chemical composition of extracts from these herbs will expand understanding of the inhibitory mechanisms involved. *Prunus mume* contains high concentrations of organic acids including 19% citric acid and 15% malic acid in the fresh fruit, and 50% citric acid and 20% malic acid in the dry fruit. *Prunus mume* also contains picric acid as a strong antifungal compound. *Rhus chinensis* contains 50–78% gallotannic acid and gallic acid, including 1,2,3,4,6-pen-ta-O-galloyl-β-D-glucose, 3-O-digalloyl-1,2,4,6-tetra-O-galloyl-β-D-glucose, 2-O-digalloyl-1,3,4,6-tetra-O-galloyl-β-D-glucose, 4-O-digal-oyl-1, 2,3,6-tetra-O-galloyl-β-D-glucose, 2,3-bis-O-digalloyl-1,4,6-tri-O-galloyl-β-Dglucose, 3-O-trigalloy-1,2,4,6-tetra-O-galloyl-β-D-glucose, 3,4-bis-O-digalloyl-1, 2,6-tri-O-galloyl-β-D-glucose, 2,4-bis-O-digalloyl-1, 3,6-O-g alloyl-β-D-glucose, and other compounds. This implies that natural organic acids including citric acid, malic acid, and gallotannic acids in Chinese medicinal herbs may have important roles for controlling Verticilium wilt in cotton. This paper provides some useful information to assist integrated disease management for Verticillium wilt of cotton in the future.

Conclusions

We conclude that the Chinese medicinal herbs tested here showed effective antifungal potential for plant disease control, and also promoted growth of cotton plants. Chinese medicinal herbs are cheap to produce, safe to use, and environmentally non-hazardous, so they have potential for use against Verticilium wilt of cotton. The results of our study offer insights on the alternative organic basis for Verticilium wilt management, and improved the cotton quality from sustainable crop production, that is likely to benefit crop farmers and managers.

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